Methods to Identify and Characterize Developmental Neurotoxicity for Human Health Risk Assessment. III: Pharmacokinetic and Pharmacodynamic Considerations

David C. Dorman,[1] Sandra L. Allen,[2] Janusz Z. Byczkowski,[3] Luz Claudio,[4] J. Edward Fisher Jr.,[5] Jeffrey W. Fisher,[6] G. Jean Harry,[7] Abby A. Li,[8] Susan L. Makris,[9] Stephanie Padilla,[10] Lester G. Sultatos,[11] and Beth E. Mileson[12]

[1]Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina, USA; [2]Zeneca Central Toxicology Laboratory, Macclesfield, Cheshire, United Kingdom; [3]Consultant, Fairborn, Ohio, USA; [4]Mount Sinai School of Medicine, Division of Environmental and Occupational Medicine, New York, New York, USA; [5]Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Rockville, MD, USA; [6]Department of Environmental Health Science, University of Georgia, Athens, Georgia, USA; [7]Neurotoxicology Group, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA; [8]Metabolism and Safety Evaluation–Newstead Laboratory, Monsanto Company, St. Louis, Missouri, USA; [9]Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency, Washington, DC, USA; [10]Neurotoxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA; [11]Department of Pharmacology and Toxicology, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, New Jersey, USA; [12]ARCADIS Geraghty & Miller, Millersville, MD, USA

We review pharmacokinetic and pharmacodynamic factors that should be considered in the design and interpretation of developmental neurotoxicity studies. Toxicologic effects on the developing nervous system depend on the delivered dose, exposure duration, and developmental stage at which exposure occurred. Several pharmacokinetic processes (absorption, distribution, metabolism, and excretion) govern chemical disposition within the dam and the nervous system of the offspring. In addition, unique physical features such as the presence or absence of a placental barrier and the gradual development of the blood–brain barrier influence chemical disposition and thus modulate developmental neurotoxicity. Neonatal exposure may depend on maternal pharmacokinetic processes and transfer of the xenobiotic through the milk, although direct exposure may occur through other routes (e.g., inhalation). Measurement of the xenobiotic in milk and evaluation of biomarkers of exposure or effect following exposure can confirm or characterize neonatal exposure. Physiologically based pharmacokinetic and pharmacodynamic models that incorporate these and other determinants can estimate tissue dose and biologic response following *in utero* or neonatal exposure. These models can characterize dose–response relationships and improve extrapolation of results from animal studies to humans. In addition, pharmacologic data allow an experimenter to determine whether exposure to the test chemical is adequate, whether exposure occurs during critical periods of nervous system development, whether route and duration of exposure are appropriate, and whether developmental neurotoxicity can be differentiated from direct actions of the xenobiotic. *Key words*: developmental neurotoxicity, pharmacodynamics, pharmacokinetics, physiologically based pharmacokinetic modeling, rat. — *Environ Health Perspect* 109(suppl 1):101–111 (2001).
http://ehpnet1.niehs.nih.gov/docs/2001/suppl-1/101-111dorman/abstract.html

Developmental neurotoxicity is any effect of a toxicant on the developing nervous system before or after birth that interferes with normal nervous system structure or function. Exposure to xenobiotics during development can cause adverse structural, functional, neurochemical, or behavioral effects. Studies conducted on laboratory animals to detect or characterize developmental neurotoxicity typically focus on behavioral and pathologic effects and rarely involve consideration of pharmacokinetic or pharmacodynamic processes. These studies have been used as the basis for human health risk assessment for regulatory purposes for many years (*1–3*), and are discussed in companion articles in this journal (*4,5*). The value of developmental neurotoxicity studies conducted in laboratory animals can be increased by consideration of pharmacokinetic processes (e.g., absorption, distribution, metabolism, and elimination processes) and pharmacodynamic effects (e.g., physiologic, biochemical, and molecular effects) of a chemical on the organism in study design and interpretation. We outline pharmacokinetic and pharmacodynamic data that can be used to improve risk assessment of developmental neurotoxicants. Pharmacologic data can verify or characterize exposure, refine the dose–response curve, determine chemical delivery to a target site, and identify effects on physiologic systems resulting from exposure to the study compound. These pharmacokinetic and pharmacodynamic data collected from animals may be used to replace or modify default assumptions related to dose–response assessment and to extrapolate from animal data to human scenarios, thus reducing uncertainty in risk assessment (*6*). Here we focus on studies conducted in the rat, the test species most commonly used in developmental neurotoxicity studies, although we refer to other species to illustrate pharmacologic factors that should be considered to improve experimental design of developmental neurotoxicity studies.

For several reasons, pharmacokinetic and pharmacodynamic processes often are more complicated in the developing nervous system than in the adult. For example, dynamic physiologic changes occur in the maternal–placental–fetal unit during pregnancy and in the offspring during postnatal development. Brain growth is rapid before and after birth, and access of chemicals to the brain may change as the blood–brain barrier (BBB) develops and matures. Exposure to the conceptus often depends on transfer of the toxicant from the dam to the developing animal via the circulation *in utero* and the milk during lactation. Thus, exposure to the developing nervous system is a function of the dose administered to the dam and the pharmacokinetics of the compound in the maternal, placental, and embryo/fetal (or neonatal) circulations. Changes in either maternal or offspring pharmacokinetics could therefore affect the extent to which the developing nervous system is exposed. In addition, the intrinsic vulnerability of the nervous system

Address correspondence to B. Mileson, ARCADIS Geraghty & Miller, 1131 Benfield Blvd., Suite A, Millersville, MD 21108 USA. Telephone: (410) 987-0032. Fax: (410) 987-4392. E-mail: bmileson@arcadis-us.com

Address reprint requests to S. Carter, ILSI Risk Science Institute, 1126 Sixteenth St., NW, Washington, DC 20036-4810. Telephone: (202) 659-3306. Fax: (202) 659-3617. E-mail: scarter@ilsi.org

This project was supported through a cooperative agreement between ILSI Risk Science Institute and the U.S. Environmental Protection Agency Office of Pesticide Programs, by the National Institute of Environmental Health Sciences, and by the ILSI Research Foundation.

The opinions expressed herein are those of the individual authors and do not necessarily reflect the views of their respective organizations or the sponsoring institutions. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Received 9 August 2000; accepted 20 October 2000.

to toxic agents varies with developmental stage at which exposure occurs, i.e., there are critical periods, both pre- and postnatally, for disruption of structural and functional nervous system development [(*7*); for review see Rice and Barone (*8*) and Adams et al. (*9*)]. There may be a heightened sensitivity of the immature nervous system to toxic insult as a result of various developmental processes such as cell formation, migration, development of neurites, and establishment of intercellular connections. There may also be greater exposure of the immature (vs. mature) nervous system to toxic chemicals. The pharmacokinetics and toxic effects of a test compound should therefore be evaluated during the relevant period of development.

Pharmacokinetic and pharmacodynamic data might be used to address questions such as whether gestational exposure to the developing nervous system occurs *in utero*, and whether the compound is transferred to the milk, creating lactational exposure. If exposure is confirmed based on pharmacokinetic data or if neurotoxicity is observed in the developing animal, the next step may be dose characterization in the dam and offspring. To characterize exposure and dose response for study design or interpretation, a pharmacokinetic profile in the dam and embryo/fetus or pup can be generated based on available data or through pharmacokinetic modeling. The ultimate goal in risk assessment is to describe the entire exposure, target tissue dose, and toxic response continuum for more accurate extrapolation to humans (Figure 1).

Factors That Modify *in Utero* Exposure of the Developing Nervous System

Factors that determine whether prenatal exposure to a xenobiotic will cause developmental toxicity include the pharmacodynamic action of the xenobiotic on the conceptus, the prenatal developmental stage at which exposure occurs, and the concentration of the active form of the xenobiotic reaching the conceptus *in utero*.

Chemicals may produce developmental neurotoxicity directly by acting on the fetus or indirectly by inducing maternal effects. Most indirect effects result from altered delivery of oxygen, glucose, amino acids, specific coenzymes, and other substrates to the fetus. Indirect effects can arise from placental insufficiency, by direct effects on maternal glucose regulation (e.g., by hypoglycemic agents), or by alterations in maternal ventilatory control (e.g., opiate-induced hyperventilation or hypoventilation). Some xenobiotics are likely to act through more than one mechanism. For example, ethanol has specific teratogenic effects in both the embryonic and fetal period but may also alter maternal nutrition and disturb fetal acid–base balance (*10*).

The nature and incidence of developmental effects of chemicals depend on the developmental stage insulted (*11*). In general, exposures to certain chemicals during predifferentiation early in gestation do not produce congenital malformations but instead lead to embryonic death or abortion (*12*). Similarly, the embryo is highly susceptible to teratogenic insult during organogenesis because organ development is continual within this period (*11*). After organogenesis, the developing nervous system can remain susceptible to chemical insult (*8,13*). Although major brain structures are largely completed during organogenesis, neuronal migration occurs and neuronal connections continue to be formed through early neonatal life; thus, brain organization is an important consideration during the fetal and postnatal periods (*14*).

The concentration of the active form of a xenobiotic reaching the conceptus *in utero* is another important determinant of effect and is influenced by maternal and fetal metabolism. Numerous xenobiotics are known to exert their toxicities only after metabolic activation by various enzymes within the body [reviewed by Juchau (*15*)]. Biotransformation may vary among species or individuals based on genetic or physiologic variables such as pregnancy, disease, or chronic drug therapy. Therefore, establishing whether metabolism is qualitatively and quantitatively similar among different species is important when making interspecies extrapolations. For example, differing susceptibilities of various inbred mouse strains, and possibly human individuals, to the developmental effects of phenytoin may involve genetically determined differences in phenytoin biotransformation (*16,17*), and different sensitivities to retinoid developmental toxicity among species can be attributed partly to differences in metabolism (*18*).

The activity of hepatic microsomal drug-metabolizing enzymes increases during human pregnancy, leading to an increase in hepatic clearance of certain drugs and chemicals (*19*). Conversely, the hepatic microsomal metabolizing enzyme activity in rats decreases during gestation to approximately 50% of control levels [for review see Oesterheld (*20*)]; however, the total metabolic capacity of the rat liver may remain constant or can increase during pregnancy because liver hypertrophy leads to increased (~40%) liver weights in pregnant rats (*21*). The human fetus possesses a well-developed complex of drug-metabolizing enzymes, albeit less active than those of the adult, whereas fetuses of common laboratory animals may be deficient in drug-metabolizing activity (*22–28*). This difference between human and rat fetus enzyme activity may be a disadvantage of the rat model. Even though levels of hepatic and extrahepatic cytochrome P450 enzymes are lower in the rat fetus than in the dam, some investigators have hypothesized that these levels may be sufficient to catalyze deleterious biotransformation reactions (*29*). Fetal tissues and placenta are deficient in A-esterases and carboxylesterase (*30*), two enzymes that may limit detoxification of organophosphate and pyrethroid insecticides in the fetus.

Plasma protein binding is another important determinant of xenobiotic disposition and action. Significant changes in plasma protein composition can occur during pregnancy (*31,32*). Both albumin and α-1 acid glycoprotein fractions are reduced during pregnancy, and the pharmacokinetics of highly protein-bound acidic and basic drugs are affected. Plasma protein levels of the developing offspring undergo changes during development (*33*) that may lead to increased levels of free (i.e., protein-unbound) drugs. Differences in the degree of protein binding between maternal and umbilical cord plasma have been demonstrated. In some cases these differences in plasma protein binding may be sufficient to account for a difference in total drug concentration between maternal and fetal plasma (*32*). Xenobiotic binding to fetal or neonatal plasma proteins may displace endogenous substrates that are normally bound to these proteins. For example, the competitive displacement of bilirubin from

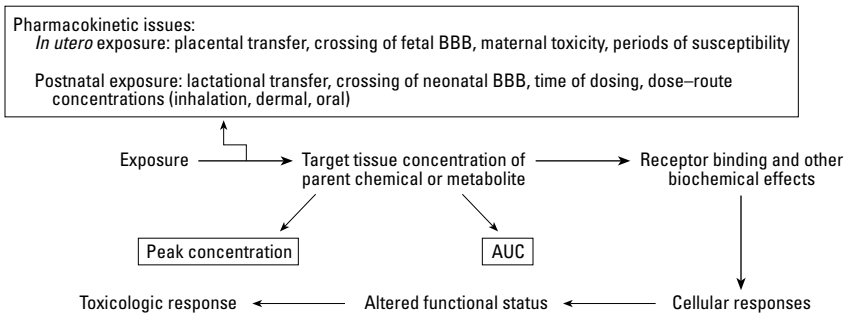

Figure 1. Overview of pharmacokinetic processes that occur during nervous system development. AUC represents the area under the concentration–time curve.

plasma proteins by diazepam, salicylates, and other drugs is one cause of clinical jaundice in infants (*31,34,35*).

Most foreign chemicals that gain access to the conceptus after maternal exposure do so through placental transfer (*36,37*), although some chemical exposure can occur before implantation. Factors that influence the rate of chemical transfer across the placenta are the thickness and surface area of the placental membrane, placental blood flow, molecular size and lipid solubility of the chemical, plasma protein binding of the chemical, and respective pHs of the maternal and fetal circulation [for review see Pratt (*38*)]. Most xenobiotics cross the placenta by passive diffusion and do so in accordance with their solubility characteristics (*39*). Low molecular-weight, lipophilic chemicals (e.g., many pesticides) are fully capable of crossing the placental membranes, and their rate of transfer is limited by placental blood flow. The placental transfer of anticholinesterase pesticides provides one well-documented example of this process (*40–45*). In contrast, high molecular-weight (> 1,000 Da) or ionized, hydrophilic compounds cross the placenta very poorly or not at all, depending on their molecular radii and the presence or absence of charged functional groups. They also cross the BBB at a rate slower than the rate of delivery by the bloodstream. This process is called membrane-limited transfer, and the constitution of the membranes determines the rate of transfer.

There are marked interspecies differences in types of placenta, orientation of exchanging vessels, and number of exchanging layers (*46*). Although these differences do not play a dominant role in the placental transfer of most drugs and chemicals, large species differences have been shown for placental permeability of hydrophilic molecules. For example, in sheep there is no significant diffusional flux for hydrophilic compounds with a molecular weight up to 400 Da. In the guinea pig, however, there is no restriction in diffusion for molecules with weights up to 5,000 Da. The permeability of human placenta is considerably higher than sheep placenta and is more comparable to guinea pig placenta (*47*).

The observed species differences in placental transfer of hydrophilic xenobiotics are caused predominantly by structural differences among placenta (Table 1). Both the human and rat placentae are hemochorial—that is, the fetal chorionic villi bathe in lacunae of maternal blood. In the human placenta, the villous capillary is separated from maternal blood by three layers of cells (*48*). The layers of the rodent chorionic villus are similar; however, they have an additional layer of cytotrophoblasts (*48*). The villous nature of the human placenta produces a haphazard arrangement that promotes a cross-current exchange between the maternal and fetal circulations. In rodents the flow system is mainly countercurrent, producing more efficient exchange. The high permeability of the human and guinea pig placenta, compared with the sheep placenta, may occur because the human and guinea pig have a thin hemochorial type of placenta, whereas the sheep has a epitheliochorial placenta in which more tissue layers separate the maternal and fetal bloodstreams. In the human hemochorial placenta, the trophoblast and the endothelium are responsible for the diffusional resistance to hydrophilic compounds. The trophoblastic components determine the overall diffusion barrier, and intercellular spaces in the endothelium restrict the diffusion of larger molecules. Trophoblasts within the placenta also express P-glycoprotein, which may protect the fetus from xenobiotic exposure (*49*).

Besides differences in placental anatomy, both human and rodent placentae undergo considerable changes throughout gestation as the normal developmental process proceeds (*50*). For example, enhanced blood flow to the placenta, increased fetal vasculature size, and proliferation of maternal vascular microvilli all contribute to increased efficiency of transplacental transport in guinea pigs during late gestation (*51*). The enhanced placental transfer of 13-*cis*-retinoic acid observed in rodents during later stages of gestation has been attributed to such maturational changes (*52*).

Several enzymes that can metabolically activate or deactivate certain compounds have been found in the human placenta as well as in the embryo and fetus, although in amounts lower than those found in the mother (*37,53*). As discussed by Hakkola et al. (*37*), relatively little is known about the role of placental metabolism in modulating prenatal developmental toxicity, but the levels of these metabolizing enzymes can change throughout gestation. Although the overall contribution of these enzymes to maternal pharmacokinetics is probably negligible, intrauterine activation of chemicals might contribute to toxic outcomes in the embryo or fetus (*54,37*).

Factors That Modify Postnatal Exposure of the Developing Nervous System

Once parturition occurs, exposure of offspring to a test chemical and or its metabolites generally occurs through the milk. Although some preformed, endogenous, systemic substances are found in milk (*55*), a selective blood–milk barrier exists for the mammary ducts of most species studied. Various factors affect the entry of compounds into breast milk, including molecular size, lipophilicity, and pH [for review see Wilson (*56*) and Wilson et al. (*57*)]. The transfer of drugs, chemicals, and micromolecular nutrients from blood to the milk occurs via mechanisms similar to transport mechanisms of other membranes. Such mechanisms include diffusion through water-filled pores, diffusion of lipid-soluble compounds through lipid membranes, and active, or carrier-mediated, transport. Passive diffusion is affected mainly by the chemical or drug disposition in lactating mothers, the physicochemical properties of the molecule, and the protein and lipid content of breast milk. In general, lipophilic chemicals penetrate membrane barriers easily and are preferentially concentrated in the milk fat globules, which can lead to a high ratio of chemical in milk compared to plasma. Because lipophilic compounds partition into fat, the fat content of breast milk is a major determinant of the chemical level in whole milk. The concentration of fat and other nutrients in the milk varies among species. Mature human milk contains approximately 3–5% fat, 0.8–0.9% protein, 6.9–7.2% carbohydrate calculated as lactose, and 0.2% mineral constituents expressed as ash (*58*). The concentration of protein (8–12%), fat (11–15%), iron, and copper in rat milk is higher than that found in human breast milk (*59*). Within each species, fat and other nutrient concentrations vary throughout lactation period postprandially and diurnally (*59*).

The pK_a of a weak acid or base is a primary determinant of its ability to enter

Table 1. Placental morphology and classification in humans and common laboratory animals.

	Tissue layers									
Placental type	mb	mce	mct	me	is	tr	fct	fce	fb	Species
Epitheliochorial		+	+	+		+	+	+		Sheep, pig
Endotheliochorial		+	–	–		+	+	+		Dog, cat
Hemochorial										
mono		–	–	–		+	+	+		Human, guinea pig
di		–	–	–		++	+	+		Rabbit
tri		–	–	–		+++	+	+		Rat, mouse

Abbreviations: +, number of cell layers; fb, fetal blood; fce, fetal capillary endothelium; fct, fetal connective tissue; is, intervillous space; mb, maternal blood; mce, maternal capillary endothelium; mct, maternal connective tissue; me, maternal epithelium; tr, trophoblast. Maternal blood is represented by a shaded column, as are intervillous space and fetal blood. The placentas of all species listed contain these layers. Columns between these layers represent cell layers (if any) that separate mb, is, and fb. Data modified from Page (*181*).

breast milk. The pH of breast milk is generally 6.6–6.8 in the human and therefore more acidic than plasma. Thus, basic compounds are often trapped in the milk and reach higher levels, whereas acidic compounds are inhibited from entry, producing lower levels. Breast milk can be considered a compartment with bidirectional transfer rather than a reservoir into which drugs accumulate. The amount of many pharmaceutical agents or drugs excreted in milk is significantly lower than the maternal dose; however, there are a number of exceptions [for review see Berlin (*60*) and Berglund et al. (*61*)]. Conversely, the amount of many chemicals or environmental agents in the offspring can often be higher than in the mother. For example, highly lipid-soluble chemicals (e.g., DDT and other organochlorine insecticides) may remain in body fat for very long periods and represent a hazard to the nursing infant (*62,63*). Metals chemically similar to calcium, such as lead, may accumulate in breast milk. These interactions could deliver significantly higher amounts of chemical to the nursing offspring than would be estimated based upon maternal levels.

Although some data describe milk levels of pharmaceutical agents, few data characterize human milk levels of environmental chemicals. Few studies reporting both milk and infant plasma levels are found in the toxicologic literature, and the validity of assumptions in predictive models for drugs has not been evaluated. Data are available from experimental animal studies, human epidemiologic studies, and case reports on the lactational transfer and uptake in the neonate of inorganic mercury, methylmercury (*64*), lead (*65*), cadmium (*66*), polychlorinated biphenyls (PCBs) (*67,68*), tetrachloroethylene (*69*), radioiodine (*70,71*), hexachlorobenzene (*72*), and other organochlorine chemicals (*73*).

The concentration of a chemical in milk depends on the rate of transfer across the epithelium and the half-life of the substance in the plasma. Chemicals with a short plasma half-life may not have time to equilibrate across the mammary epithelium, so milk concentrations may be low compared with plasma concentrations. The key starting point for estimating the amount of compound in the milk is the free concentration of drug or chemical in the maternal plasma. Total plasma concentration is used often as a surrogate for this calculation. Peak plasma concentration following single or repeated exposure is a function of the dose and dose rate, route of exposure, bioavailability, volume distribution, and amount of drug eliminated during the assimilation phase. The decline in plasma concentration after the peak is determined largely by the elimination half-life. These factors also determine the concentrations of drugs or chemicals achieved in the suckling infant, with the dose rate being the amount ingested over time in the mother's milk. However, the offspring blood levels of chemical also depend on the degree of absorption in the gastrointestinal tract, as well as distribution, metabolism, and excretion. The milk-to-plasma (M:P) ratio, which compares milk drug concentration with maternal plasma drug concentration, serves as an index of the extent of drug excretion in the milk. The impact of these various determinants has been quantified, and pharmacokinetic models have been established for predicting the behavior of unstudied chemicals (*57,74–77*).

These principles have been used successfully to describe M:P partitioning of many chemicals. However, *in vivo* studies in a variety of species have identified some drugs that are found in milk at higher-than-predicted concentrations. Drugs for which this is true include aminopyrine (*78*), N4-acetylated *p*-aminohippuric acid (*79*), N4-acetylated sulphanilamide (*80*), acyclovir (*81*), cimetidine (*82–85*), and nitrofurantoin (*86*). The unpredictable M:P ratios observed for these compounds may be due to an active transport mechanism.

An additional concern that should be considered in lactational exposure studies is the potential for the xenobiotic to alter milk secretion and composition. Such interference can occur in mammary development, secretion by mammary alveolar cells, hormonal support for lactation, or nutrient transport [for review see Neville and Walsh (*87*)].

Methods Used to Confirm Exposure of the Developing Nervous System

Several factors should be considered when designing studies to assess whether placental transfer of a chemical might occur. Single time-point measurements of either maternal or fetal chemical concentrations are difficult to interpret in developmental neurotoxicity studies for various reasons. First, some xenobiotics may be transported more readily across the placenta during late gestation than during early gestation. Second, placental transfer may vary over time, depending on the dosing paradigm used (i.e., bolus injection vs. dietary exposure). In addition, it is difficult to predict whether a bolus dose or continuous infusion of a chemical given to the dam will lead to a higher concentration within the embryo/fetus; either may produce a higher exposure, depending on the substance administered. Substances that are eliminated rapidly from the maternal circulation tend to lead to fetal plasma concentrations that are only a fraction of the maternal peak concentration.

Current research techniques to measure placental transfer include the use of the chronically cannulated sheep, rabbit, or monkey, the isolated perfused human placenta or placental lobule, and the small-animal placenta (rabbit, guinea pig, and even rat) perfused *in situ* to determine transfer rates for chemicals (*88*). Concentration gradients across the sheep placenta are often larger than in primates, especially for less lipid-soluble chemicals (*89*). The large litter size of rabbits allows individual fetuses to be removed in sequence to measure chemical concentrations in both plasma and tissue (*90,91*). Small laboratory animals may be used to characterize distribution of the toxicant, with either autoradiography (*92*) or extraction and chemical assay (*91*) used to detect the toxicant.

The concentration of xenobiotic in the fetus may be estimated from the placental transfer rate and the rate of direct elimination by the fetus using the concentration-time course of the chemical in the mother. Depending on the particular xenobiotic, pharmacokinetic end points of interest can include comparisons of peak (or trough) fetal and maternal concentrations, area under the concentration-time curve (AUC), and mean steady-state concentrations in the fetus and dam expressed as a fetal-to-maternal ratio.

A transplacental binding gradient may account partly for the differences in placental transfer and teratogenic potency between *all-trans* and 13-*cis*-retinoic acid in the mouse (*93*). A pH gradient across the placenta also appears to play a role in the extent of placental transfer of some substances. The embryonic compartment is alkaline relative to maternal plasma during early rodent organogenesis, and several teratogenic weak acids can accumulate in the embryo during this period, presumably by ion trapping (*94*). This concept has been formalized in a physiologically based pharmacokinetic (PBPK) model (*95*).

Experimental animal studies evaluating placental (and lactational) transfer often rely on the use of high-purity radiolabeled chemicals to determine xenobiotic distribution patterns in the dam and fetus or neonate. The use of radiolabeled chemicals also facilitates mass balance determinations. Measurement of radiochemicals often relies on whole-body autoradiography techniques using small numbers of animals. Because individual radiolabeled isotopes are followed, interpretation of these studies requires some knowledge of the metabolism to ensure that the radiolabel remains with the metabolite of interest. Studies examining the transfer and distribution of nonradiolabeled chemicals require the development of sensitive analytic methodologies to evaluate the parent compound and its metabolites.

Exposure to the developing animal *in utero* can be confirmed using analytic techniques to measure the parent compound or active

metabolite in plasma, blood, or amniotic fluid. Evidence of exposure in nursing neonates can be demonstrated by determining the concentration of the test substance and its important metabolites in pup blood or tissue during the lactation period or by measuring chemical-specific biomarkers of exposure or effect in pups (e.g., cholinesterase inhibition or carboxylesterase inhibition). The measurement of toxicant concentrations in milk is often used as a surrogate to confirm neonatal exposure. Changes in the magnitude of exposure over time can be estimated by sampling either the maternal milk or stomach contents of the pups at several time points during the lactation period. Each of these sampling procedures is hindered by the preference for a terminal sacrifice of the experimental animal for adequate sampling. An alternative approach is to employ cross-fostering in the experimental design to distinguish between body burden of a drug or chemical resulting from gestational or lactational routes of exposure. Exposure in the developing animal can be confirmed by measuring a biomarker of exposure or effect rather than the actual toxicant.

Characterization of Exposure

In studies where only maternal animals are treated and there is little or no opportunity for other types of postnatal exposure (such as in a gavage study), exposure in pups is mediated by the presence of the test substance or its metabolites in milk, the presence of normal lactation function and behavior on the part of both dam and pups, and the volume of milk consumed by the pups. In this type of treatment scenario the amount of test substance delivered to the pups via the milk does not remain constant because levels in milk may change over time (*96*), and milk intake decreases in late lactation.

Some other types of exposure in the pups can occur in early or late lactation, depending on the study design and route of administration. For instance, pups could be exposed to the test substance by ingesting the material from the maternal skin if it has been dermally applied to the dams (or if there is residual material on dams dosed by inhalation in a whole-body chamber), by dermal exposure to treated diet, by consumption of maternal feces, or by oral exposure to treated feed as pups approach the age of weaning and begin to consume solid food. These postnatal exposures are difficult to quantify. Some of the postnatal offspring exposures, even when delivered via multiple mechanisms, may be significantly lower than maternal exposures, whereas others (e.g., milk plus treated feed during late lactation) may be significantly higher.

Numerous pharmacokinetic processes in both the maternal animal and offspring mentioned above (absorption, distribution, placental and lactational transfer, protein binding, metabolism, and excretion) determine the critical target-organ concentrations of an active agent. These processes can vary depending upon the delivered dose, route of administration, duration of exposure, gestational stage, and species. Therefore, characterization of exposure is a critical element in defining the toxicologic dose–response (or concentration–response) relationship for proper interpretation and extrapolation of study results. For example, placental transfer of the antiepileptic drug phenytoin varies significantly among animal species as well as with gestational age and route of administration (*97*). The gestational stage-dependent kinetics of the herbicide 2,4,5-T in pregnant mice would be expected to produce increasing embryo or fetal exposures over the course of pregnancy at the same maternally administered dose (*98*). The dose-dependent kinetics of the developmental neurotoxicants phenytoin and valproic acid appear to be accentuated in pregnant animals (*99,100*). These findings indicate that maternally administered dose may be a poor surrogate for concentrations in the developing organism and emphasize the importance of exposure monitoring in the evaluation of developmental neurotoxicity.

Exposure characterization is particularly important in extrapolating study results to humans. In some cases peak exposure is linked to an adverse effect, and in other cases the total exposure over time is linked to the adverse effect; thus, different pharmacokinetic parameters may be important in predicting outcome, depending on the study compound (*101,102*). For example, maximal maternal plasma and embryo concentrations (Cmax) correlate with the development of neural tube defects in animals treated with the neuroteratogen valproic acid (*103*). Conversely, the AUC values for retinoids correlate best with their teratogenic activity (*104,105*). There is some evidence that peak concentrations may play a crucial role in the neurobehavioral effects of early lead exposure in monkeys (*106*). For some developmental toxicants such as 2-methoxyethanol, the critical pharmacokinetic parameter (e.g., Cmax vs. AUC) apparently depends on the organ system and developmental period during which exposure occurs (*107*). These data suggest that the pattern of exposure is a key determinant of developmental toxicity, which could have important implications for risk assessment in humans (*108,109*).

The level of exposure characterization may be limited by practical considerations in a routine developmental neurotoxicity study but ideally could at least include quantification of the test compound (and any major metabolites) in maternal plasma over time. However, more proximate measures of offspring exposure may be essential for some compounds. For example, lactational exposure following administration of the same maternal dose of methylmercury or inorganic mercury to mice produced much higher brain mercury concentrations in the pups of dams given methylmercury despite similar pup plasma mercury concentrations in the two groups (*110*). Thus, plasma-level data must be viewed in the context of the disposition of a compound within the organism, and all the pharmacokinetic processes that determine the concentrations of the active substance at the target must be considered. Although such information is most useful if analogous data are available for humans, a lack of human data does not preclude its use. For example, it can be incorporated into physiologically based models to improve the estimation of target organ exposure in humans. The concept of using PBPK models to better describe the disposition of a xenobiotic in both rodents and humans has been discussed in the context of developmental risk assessment by Welsch et al. (*111*) and is discussed in more detail later in this article.

Biochemical Markers of Exposure and Effect in Developing Animals

A biomarker of effect can be defined as a measurable biochemical or physiologic alteration within an organism recognized to have a potential impact on health (*112*). A biomarker of exposure may be a predictive indicator that does not impact health. Examination of biomarkers of effect following exposure of a laboratory animal to a neurotoxicant can be used to identify the lowest dose capable of eliciting the potential adverse effect and provide information on the mechanism of action of the toxicant. A number of biomarkers have been used to identify insult in the adult nervous system, including astrocyte expression of glial fibrillary acidic protein (GFAP), induction of stress proteins, increased density of apoptotic cells, increased neuronal degeneration, and alterations in receptor density or function throughout the brain (*113,114*) [for review, see Costa (*115*)]. The applicability of studies characterizing biomarkers of effect in the developing nervous system depends on a clear understanding of the differences between juvenile and adult brains and adequate documentation and validation studies. Therefore, the utility of the methods and techniques used to identify specific biomarkers of effect in the adult brain to evaluate the toxicity of compounds on the nervous systems of developing animals remains to be seen.

Analysis of potential biomarkers of exposure or effect in the developing nervous system will be complicated by the rapid brain growth and neuronal development that occur during pre- and postnatal periods. Some of the biomarkers of effect in adults may occur normally in the developing brain as a result of basic processes that underlie nervous system growth and development. These processes—including cell replication, migration, and differentiation; apoptosis; myelination of neurons; and synapse formation—occur in a precisely ordered sequence in each region of the brain, both *in utero* and postnatally, and can be identified by molecular markers. Exposure to a neurotoxicant may alter the pattern of brain development, or the normal pattern of development may be shifted in time as a result of such exposure.

The biochemical and structural changes that occur in the normal developing nervous system are enormous. Markers of effect of low specificity (e.g., brain weight determinations) may be useful for evaluating toxicant actions in the developing nervous system but are less useful in evaluating toxicant actions in the adult nervous system. For example, measures of brain weight, protein or lipid concentration, and RNA or DNA content all produce simple but accurate measures of brain development and the schedule of that development either in the whole brain or selected regions. More dynamic measures include incorporation of precursors into macromolecules such as proteins, lipids, RNA, or DNA, indicating both amount and turnover in nervous system tissue (*116*). Alterations in these biochemical parameters may signal either a change in pattern of brain development or a shift in time of development. Distinguishing between these two situations using biochemical measures can be a challenge. A description of the normal or baseline pattern of ontologic expression of the biochemical target throughout development should be available before any of the methods to evaluate biochemical markers of effect described above are used for routine analysis of developmental neurotoxicant action. For example, an evaluation of changes in protein or lipid content in a given brain region will not be useful for assessing developmental neurotoxicity until an analysis of control brain protein or lipid content at specific time points is available.

Assays to evaluate more specific biochemical and pharmacologic markers of exposure or effect can be conducted using brain tissue homogenate preparations or histologic sections. Examples of this approach include measures of specific protein or mRNA expression used as sentinels of appropriate brain development: myelin gene expression and undernourishment (*117*); muscarinic receptor expression and parathion exposure (*118*); astroglial markers and cocaine exposure (*119*); neurogranin and myelin basic protein expression and exposure to PCBs (*120*); and chemical-induced hypothyroidism (*121*). Tissue homogenates can be assayed to determine the levels of neurotransmitters, receptor density, or receptor affinity in given regions of the brain. The function of these receptors may also be assessed by analyzing the second messenger activity linked to a given receptor type in tissue homogenates, such as adenylate cyclase activity or ion flux linked to receptor activation (*122,123*). Many novel histologic methods have been developed that allow detection and characterization of alterations in specific regions of the brain. Among the potentially useful methods for identifying and localizing biochemical markers of effect in the developing animal brain are silver stain for identification of neuronal degeneration, and terminal deoxynucleotidyl transferase-mediated deoxyuridine diphosphate nick-end labeling for detection of apoptotic cells. Immunohistochemical labeling of GFAP, heat-shock proteins, and other developmentally regulated proteins can also be assessed (*124*). One must always be aware that the sensitivity for many of these measures may be much lower in the developing brain than in the adult because of the dynamic and rapidly changing events occurring in the normal development of the nervous system.

Microarray technologies offer a new approach for examining alterations in gene expression following chemical exposure (*125*). This approach allows determination of the presence or absence of particular nucleotide sequences or the relative abundance of an mRNA species in one sample compared with another sample. The application of this technique most relevant to toxicity testing is in determining the mRNA expression patterns of numerous genes at a single time. Although it is tempting to think of this procedure as one for the high throughput of samples, this technology instead provides a wealth of information on a limited number of samples. From such information, new hypotheses can be developed and subsequently tested with more traditional techniques such as reverse transcriptase-polymerase chain reaction. Given the vast number of genes regulated during development, each according to a distinct developmental temporal pattern of expression, choosing any one of two (or more) ages at which to examine chemical-induced alterations in development is difficult. Measurements conducted at one developmental stage cannot distinguish between an increase or decrease in a gene or simply a change, delay, or acceleration in the normal developmental profile. Thus, the need to generate patterns of gene expression during the course of development will require technologic advancements before routine evaluation of larger numbers of samples becomes feasible. Even when these technical limitations are overcome, changes in normal gene expression during development may eclipse the ability to detect toxicant-induced effects.

Application of Dosimetry Modeling to Developmental Neurotoxicology

The precise characterization of dose–response relationships and the extrapolation of results from animals to humans are both issues of paramount concern in toxicology and risk assessment that may be improved by PBPK modeling. A PBPK model is a series of mathematic equations based on organism-specific and chemical-specific information that describe the pharmacokinetic disposition of a foreign chemical within an organism (*126,127*). Solution of these equations will simulate the concentration of a chemical (as well as metabolites, if desired) with time in the tissue of interest. The power of PBPK modeling therefore lies in its ability to estimate the amount of the active form of a chemical at its target site within the body over time, given virtually any exposure paradigm. Moreover, extrapolation of an animal PBPK model to humans can be attempted through substitution with human physiologic and, where feasible, human chemical-specific information. Therefore, PBPK modeling can improve our understanding of the risks of developmental neurotoxicants by estimating more precisely the exposure of the conceptus or offspring to the active form of the toxicant after maternal exposure. This approach also improves our ability to extrapolate pharmacokinetic data from animals to humans.

PBPK modeling of various chemicals in animals during pregnancy has been reported (*95,128–132*). For example, O'Flaherty et al. (*95*) developed a PBPK model in the pregnant rat and mouse for 5,5′-dimethyloxazolidine-2,4-dione (DMO) that accounted for physiologic changes that occur throughout gestation. Their model accurately simulated DMO levels in embryo plasma and embryo homogenate on gestational days 10, 11, and 13 after maternal exposure. More sophisticated models could, in theory, simulate levels of active toxicant within various tissues or portions of tissues of the conceptus. Similarly, modeling of lactational transfer in rats and humans has been successful with certain chemicals (*133,134*). Fisher et al. (*134*) developed a PBPK model for lactating women that estimated the amount of certain volatile organic chemicals ingested by a nursing infant for a given nursing schedule after a maternal occupational exposure. Important features of the model included a milk compartment that changed in volume in response to a nursing infant.

Pregnancy and lactation PBPK models can design experiments, use information related to mode of action, and identify important data gaps. Under ideal circumstances, pharmacokinetic data obtained in humans would be used to validate PBPK models developed using animal data. Few pharmacokinetic data are available for most chemicals in pregnant or lactating women, although there are exceptions for some drugs,such as antiepileptic agents that must often be administered throughout pregnancy (*135–137*). Approaches currently used for estimating human exposure have been described (*138*).

The construction of a PBPK model begins with the description of the body as a series of compartments representing individual tissues or tissue groups (Figure 2). Those tissues represented by compartments are selected for their relevance to the disposition or action of the chemical under consideration. For example, a central nervous system (CNS) depressant requires inclusion of the brain as a compartment, whereas hepatic metabolism of a drug necessitates the use of the liver as a compartment. Similarly, a gestational PBPK model requires compartments for the placenta and the conceptus (*95*), whereas a lactational model requires a milk compartment (*134*). Following selection of appropriate compartments, differential equations are written to describe the fate of a chemical as it passes through each tissue. Many examples of such equations have been published. Organism-specific parameters for which values must be supplied to the differential equations include tissue blood flow, organ volume, cardiac output, and in certain instances ventilation rate, all of which can be obtained from the literature (*139*). Chemical-specific parameters are, at a minimum, elimination rate constants and tissue:blood partition coefficients, and often must be determined experimentally. Various approaches have been used to determine these values, and the specific methodology employed depends to a large extent on the physiochemical and pharmacokinetic characteristics of the chemical under study. If metabolite disposition is to be included in the model, chemical-specific parameters for the parent compound and the metabolites of interest must be determined. Solution of the model requires a computer equipped with software that can solve simultaneous differential equations. Probably the most commonly used software for this purpose has been Advanced Continuous Systems Language (Pharsight Corporation, Mountain View, CA, USA), although many others can be used as well.

Although PBPK modeling is an extremely powerful pharmacokinetic tool, it is not without problems. Building PBPK models can require considerable resources. At a minimum, moderate programming skills are required, and tissue:blood partition coefficients and elimination rate constants can be difficult and time consuming to determine accurately for many types of chemicals. Furthermore, with a rodent gestational model, the limited amount of tissue obtained from a conceptus or the placenta requires the use of a large number of animals. Although validation of animal PBPK models can be accomplished by comparing model predictions with empirical pharmacokinetic data, such data are often missing and unobtainable in humans. An important challenge to modelers is to address these complicating issues so that PBPK modeling can become a more useful tool, routinely used in all areas of toxicology.

Practical Considerations in the Experimental Design of Pharmacokinetic Studies in Developmental Neurotoxicity

The selection of route of administration, duration of exposure, and time during nervous system development that the xenobiotic will be administered can influence whether developmental neurotoxicity occurs following xenobiotic exposure. Thus, the decision of when to initiate chemical exposure is critical. Developmental toxicity studies are performed typically by exposing pregnant animals to a test substance beginning at uterine implantation and early stages of organogenesis, including neurulation of the embryo (around gestation day 6 in rats). Several studies have identified specific malformations, especially neural tube defects, that appear to have resulted from preimplantation exposures (*140*). Additionally, there is some indication that very early gestational exposures to cholinesterase-inhibiting chemicals, while not causing such frank malformations as neural tube defects, may produce more subtle alterations in neurologic development (*141,142*). Thus, maternal dosing beginning at or before conception may be important for testing some xenobiotics. Another reason for initiating exposure before conception is that some xenobiotics require long exposure times to reach pseudo–steady-state conditions in the dam (*143*).

Postnatal exposure of animals may be critical in the design of developmental neurotoxicity studies and may depend upon the experimental question. Postnatal brain development occurs in both humans and rodents (*13,144*). For example, a recent magnetic resonance imaging study in children age 3–15 identified evidence of quantifiable spatial changes in the corpus callosum throughout prepubertal childhood (*145*). Although regional neurogenesis occurs predominantly during gestation in both humans and rats, neurogenesis of hippocampal dentate granule cells and cerebellar neurons continues into the postnatal period in rats compared to time of parturation in humans (*13*). In each species, the formation of the neural network and connections as well as myelination are significant processes that occur postnatally [for review see Jacobson (*146*)]. Continuation of dosing after birth may be necessary to ensure exposure to some chemicals during this critical period of brain development. Direct neonatal dosing may be required for developmental neurotoxicity testing of some xenobiotics if lactational transfer does not occur. Additional scenarios in which children may be exposed (e.g., inhalation, chemical

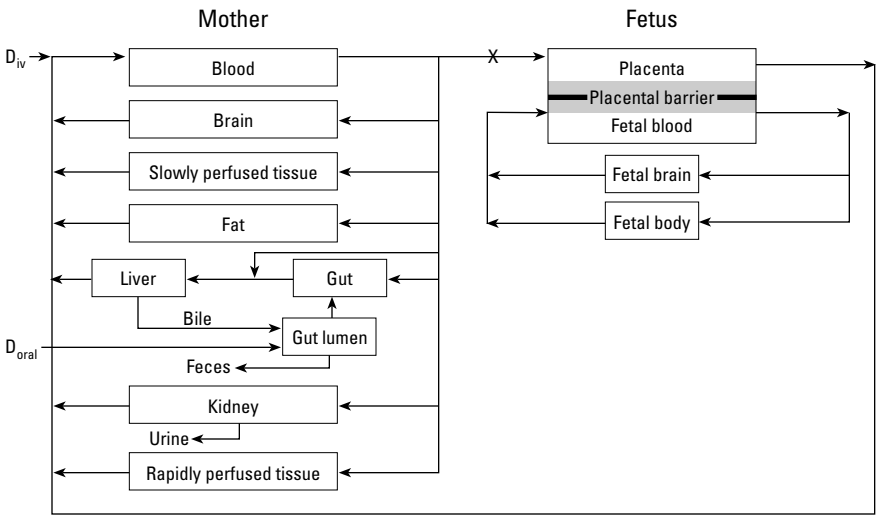

Figure 2. The conceptual framework for the PBPK model of gestational transfer of a chemical that is potentially neurotoxic from the exposed mother to the fetus. Arrows connecting tissue compartments indicate movement of chemical within the body. X denotes a time-dependent on-off switch. In this model, exposure may occur from intravenous injection or ingestion. D_{iv}, intravenous dose of chemical (mg/hr). D_{oral}, oral dose of chemical (mg/kg/day).

residues in food or water, pica) might be addressed by direct neonatal dosing studies.

Although direct dosing of pups maximizes neonatal exposure to the test compound, it is not necessarily representative of chemical exposure of the human fetus during the third trimester, which depends on the metabolism and pharmacokinetics of the chemical in the mother. Practical considerations associated with direct dosing of pups must be overcome. Logistical problems associated with dosing large numbers of pups in a study can be significant, and there are limitations in the vehicle and volume of solution that can be administered repeatedly to young animals (*147*). One additional concern is that direct oral dosing of very young pups might injure or stress the pups, which could lead to functional and behavioral effects not associated with exposure to the test chemical. A potential advantage of direct dosing of a test chemical to pups at precisely known dose levels is that this approach allows a more direct comparison of toxic response for adult and juvenile animals (*148*). In addition, if exposure is limited to the postnatal period, direct dosing of pups allows the use of interlitter dosing designs that can significantly decrease the number of animals required for any one study. This information could be useful in the assessment of age-related sensitivity to xenobiotic exposure.

Another issue to be considered is the anticipated route of human exposure. For food-use pesticides with potential dietary residues, the most appropriate administration route might be oral (gavage or dietary). In some studies dams are treated daily by gavage with a measured bolus dose of test substance that is dissolved or suspended in a nontoxic vehicle [e.g., Dorman et al. (*148*)]. In a dietary study the test substance is mixed into the feed, which is made available to the animals for *ad libitum* consumption throughout the dosing period. Consumption of the mixed feed occurs over a number of hours each day, but consumption may be affected by unpalatability of the admix. Dermal and inhalation studies are conducted with limited daily exposure periods of approximately 6 hr for the dams. Similar problems can occur with exposure via the drinking water. Both dietary and drinking water routes of exposure require substantial evaluation to characterize chemical stability in the dosing vehicle. Inhalation developmental neurotoxicity studies can be challenging because maternal separation from pups may have a negative impact on the developing nervous system and there are difficulties associated with exposing neonatal animals. Some investigators have exposed dams and neonatal pups simultaneously using bedding and stainless steel caging held within a larger, well-mixed exposure chamber (*149–151*). A whole-body exposure system that permits the simultaneous exposure of rat dams and neonatal pups has been developed recently and used for developmental neurotoxicity studies (*152,153*). In all cases, actual dose delivered to the maternal animal depends on the absorption of the chemical, which may be affected by many factors related to the physicochemical properties of the test substance or to the study design.

Fetal tissue (e.g., blood, brain) levels may confirm fetal exposure but may not be useful in determining how to optimize exposure to rat pups; there are few, if any, alternative actions to increase *in utero* exposure levels. If there is no placental transfer of the parent compound or active metabolite in the rat, an evaluation of differential metabolism may indicate whether exposure of the human fetus is likely, assuming that rat and human placental transfer are qualitatively similar. If the assumption regarding relevance of rat placental transfer to humans is questioned, either a different animal model (such as the mini pig) might be appropriate, or it may be more relevant to omit exposure during gestation and focus on exposing rat pups after birth.

Rapid changes in organ development and function occur during the neonatal period. The low body weight of neonates compared to adults and the high food consumption per kilogram of body weight mean that tissue levels of test chemicals can reach higher levels in newborns than in adults. Furthermore, the pharmacokinetics of many chemicals are age-specific. For example, mercury, manganese, and many other neurotoxic metals have higher gastrointestinal absorption and less effective renal or biliary excretion in neonates than in adults (*154,155*).

The metabolic capacity of the newborn animal also differs from that observed in adults. For example, postnatal animals may be deficient in cytochrome P450 enzymes, A-esterases, and carboxylesterase. The deficiency in P450 enzyme activity could protect the young animal from toxicants activated by P450 because the activation potential is less in the young animal than in the adult. This may not be the case for organophosphorus pesticide toxicity because both the activation and detoxification potential in young animals usually is less than in adults (*156*), producing increased toxicity in the young (*157*). This increased toxicity stems mainly from an age-related deficiency in detoxification enzymes in the young (*158–160*).

The adult nervous system is protected from many toxic substances circulating in the blood by the BBB and blood–nerve barriers. For this reason, there have been efforts to deregulate possible neurotoxicants according to their expected inability to enter the nervous system (*161*). However, such assumptions are problematic in attempts to predict the potential neurotoxicity of a chemical on the developing nervous system at a time when the BBB may not be fully functional. Even in adults, the BBB does not always protect the brain from the passage of toxicants because this barrier is permeable to many xenobiotics. In addition, the BBB is in a dynamic state, with altered permeability occurring during physiologic and pathologic conditions. Besides developmental and pathophysiologic regulation of the BBB, chemical transport into the various brain regions can display regional differences. For example, systemically administered glutamate causes neurotoxicity in the circumventricular organs and other areas of the CNS that are least protected by the BBB (*162*).

The timing of BBB development is a matter of much controversy, partly because the different markers used to define the barrier appear at different times during development (*163,164*). Furthermore, the timing of BBB development varies among animal species. For example, the BBB is not fully developed during fetal life in rodents; however, complex tight junctions in endothelial cells are present very early in human fetal development (*165*). Species differences in functional aspects of the BBB remain to be determined. The BBB marker P-glycoprotein also appears very early in brain endothelial cells, indicating advanced differentiation even at the earliest stages of development (*166*). In contrast, astrocytic foot processes and the basement membrane develop postnatally. The relatively high permeability of fetal and neonatal BBB has been ascribed to the uptake of plasma solutes by endothelial cells through a vesicular pathway (*165*). Although controversial because of possible artifacts associated with the use of high volumes of intravascular tracers in developing animals, the BBB apparently does not reach the stage of impermeability seen in adults until some time postnatally, thus increasing susceptibility to blood-borne neurotoxicants (*164,167–169*).

The level of maturity of the BBB is not the only factor that determines the transport of neurotoxicants into the brain. The physicochemical characteristics of blood-borne compounds are also important determinants in their capacity to cross the BBB. Lipophilicity plays a major role in determining the rate at which xenobiotics may enter the CNS compartment. Once inside the CNS, a highly lipophilic substance tends to accumulate because of the high lipid content of myelin and other neural structures. Thus, the extent of exposure of the CNS to a lipophilic toxicant would be higher than the systemic exposure of the organism (*170*). An ionized compound will not enter the CNS as readily as a compound that is not ionized, in

which case it will enter the brain at a rate proportional to its lipid:water partition coefficient (*171*). For example, methyl mercury enters the brain much more readily than inorganic mercury (*110,172*).

Besides lipophilic substances, other substances that structurally resemble nutrients normally taken up by the CNS can gain facilitated or receptor-mediated access through specific transport mechanisms (*173,174*). Some of these mechanisms normally transport hormones, amino acids, peptides, proteins, fatty acids, and trace elements (*175*). One such transporter mechanism, the transferrin system, is present on the surface of BBB endothelial cells. The transferrin receptor complex transports iron and manganese (*176,177*). Areas with high levels of iron receive input from areas with high levels of transferrin receptor in the vasculature and neuropil. For example, the iron-rich areas of the substantia nigra and globus pallidus receive input from the nucleus accumbens and caudate putamen, areas rich in transferrin receptors (*178*). Transferrin and its receptor may also transport other cations such as aluminum (*179*). This mode of access of neurotoxic metals into the CNS may also explain earlier observations of enhanced lead absorption during iron deficiency and anemia (*180*).

Conclusions

Our goal was to review pharmacologic factors that should be considered to improve the design and interpretation of developmental neurotoxicity studies. Many of the pharmacokinetic methods, analytic chemical techniques, and modeling approaches needed to evaluate the pharmacokinetics of a xenobiotic in the developing organism are available to the research investigator. The systematic application of these resources to developmental neurotoxicology remains in its infancy. Our intent was to emphasize the value of collecting pharmacokinetic and pharmacodynamic data in developmental neurotoxicity studies to improve the risk assessment of xenobiotics. Our hope is that this information will inform and stimulate the reader to consider the following questions when designing studies to evaluate potential developmental neurotoxicity of a xenobiotic:

- Will the exposure to the test chemical *in utero* and postnatally be confirmed or characterized adequately?
- Are the appropriate critical periods of nervous system development considered in the study design?
- Are the route and duration of exposure appropriate to detect developmental neurotoxicity in laboratory animals relevant to humans?
- Was the experiment designed so as to evaluate direct actions of the chemical separately from developmental neurotoxicity?
- Are there data to suggest that species or life-stage differences in xenobiotic pharmacokinetics or biologic response may occur in developing animals following exposure?

References and Notes

1. Bignami G. Economical test methods for developmental neurobehavioral toxicity. Environ Health Perspect 104(suppl 2):285–298 (1996).
2. Ulbrich B, Palmer A. Neurobehavioral aspects of developmental toxicity testing. Environ Health Perspect 104(suppl 2):407–412 (1996).
3. Tilson H. The role of developmental neurotoxicity studies in risk assessment. Toxicol Pathol 28:149–156 (2000).
4. Cory-Slechta DA, Crofton KM, Foran JA, Sheets LP, Ross JF, Weiss B, Mileson BE. Methods to identify and characterize developmental neurotoxicity for human health risk assessment I: Behavioral effects. Environ Health Perspect 109(suppl 1):79–91 (2001).
5. Garman RH, Fix AS, Jortner BS, Jensen KF, Hardisty JF, Claudio L, Ferenc S. Methods to identify and characterize developmental neurotoxicity for human health risk assessment II: Neuropathology. Environ Health Perspect 109(suppl 1):93–100 (2001).
6. Moore JA, Daston GP, Faustman E, Golub MS, Hart WL, Hughes C Jr, Kimmel CA, Lamb JC IV, Schwetz BA, Scialli AR. An evaluative process for assessing human reproductive and developmental toxicity of agents. Reprod Toxicol 9:61–95 (1995).
7. Rodier PM. Developmental neurotoxicology. Toxicol Pathol 18:89–95 (1990).
8. Rice DC, Barone S. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect 108(suppl 3):511–533 (2000).
9. Adams J, Barone S Jr, LaMantia A, Philen R, Rice D, Spears L, Susser E. Workshop to identify critical windows of exposure for children's health: neurobehavioral work group summary. Environ Health Perspect 108(suppl 3):535–544 (2000).
10. West J, Chen W , Pantazis N. Fetal alcohol syndrome: the vulnerability of the developing brain and possible mechanisms of damage. Metab Brain Dis 9:291–322 (1994).
11. Schardein, JL. Chemically Induced Birth Defects. New York:Marcel Dekker, Inc., 1993.
12. Rutledge JC. Developmental toxicity induced during early stages of mammalian embryogenesis. Mutat Res 396:113–127 (1997).
13. Bayer SA, Altman J, Russo RJ, Zhang X. Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. Neurotoxicology 14(1):83–144 (1993).
14. Chriboga C. Fetal effects. Neurol Clin 11:707–728 (1993).
15. Juchau MR. Bioactivation in chemical teratogenesis. Annu Rev Pharmacol Toxicol 29:165–187 (1989).
16. Finnell RH, Chernoff GF. Gene-teratogen interactions. An approach to understanding the metabolic basis of birth defects. In: Pharmacokinetics in Teratogenesis (Nau H, Scott WJ, eds). Boca Raton, FL:CRC Press, 1987;97–109.
17. Buehler BA, Delimont D, Van Waes M, Finnell RH. Prenatal prediction of risk for the fetal hydantoin syndrome. N Engl J Med 322:1567–1572 (1990).
18. Kraft JC, Slikker W Jr, Bailey JR, Roberts LG, Fischer B, Wittfoht W, Nau H. Plasma pharmacokinetics and metabolism of 13-cis- and all-transretinoic acid in the cynomolgus monkey and the identification of 13-cis- and all-transretinoyl-beta-glucuronides: a comparison to one human case study with isotretinoin. Drug Metab Dispos 19:317–324 (1991).
19. Tanaka E. Gender-related differences in pharmacokinetics and their chemical significance. J Clin Pharm Ther 24(5):339–346 (1999).
20. Oesterheld JR. A review of developmental aspects of cytorcrome P450. J Child Adolesc Psychopharmacol 8(3):161–174 (1998).
21. Symons A, Turcan R, Parke D. Hepatic microsomal drug metabolism in the pregnant rat. Xenobiotica 12:365–374 (1982).
22. Pelkonen, O. Transparental transfer of foreign compounds and their metabolism by foetus. In: Progress in Drug Metabolism, 2 (Bridges JW, Chasseaud LF, eds). New York:John Wiley, 1977;119–161.
23. Pelkonen O. Biotransformation of xenobiotics in the fetus. Pharmacol Ther 10:261–281 (1980).
24. Raucy JL, Carpenter SJ. The expression of xenobiotic-metabolizing cytochromes P450 in fetal tissues. J Pharmacol Toxicol Methods 29:121–128 (1993).
25. Juchau MR, Chao ST, Omiecinski CJ. Drug metabolism in the human fetus. Clin Pharmacokinet 5:320–339 (1980).
26. Kitada M, Kamataki T. Cytochrome P450 in human fetal liver: significance and fetal-specific expression. Drug Metab Rev 26:305–323 (1994).
27. Miller MS, Juchau MR, Guengerich FP, Nebert DW, Raucy JL. Symposium overview: drug metabolic enzymes in developmental toxicology. Fundam Appl Toxicol 34:165–175 (1996).
28. Goodman DR, James RC, Harbison RD. Placental toxicology. Food Chem Toxicol 20:123–128 (1982).
29. Juchau MR, Lee QP, Fantel AG. Xenobiotic biotransformation/bioactivation in organogenesis-stage conceptal tissues: implications for embryotoxicity and teratogenesis. Drug Metab Rev 24:195–238 (1992).
30. Lassiter TL, Padilla S, Mortensen SR, Chanda SM, Moser VC, Barone S. Gestational exposure to chlorpyrifos: apparent protection of the fetus? Toxicol Appl Pharmacol 152:56–65 (1998).
31. Notarianni LJ. Plasma protein binding of drugs in pregnancy and in neonates. Clin Pharmacokinet 18:20–36 (1990).
32. Perucca E, Crema A. Plasma protein binding of drugs in pregnancy. Clin Pharmacokinet 7:336–352 (1982).
33. Ehrnebo M, Agurell S, Jalling B, Boreus LO. Age differences in drug binding by plasma proteins: studies on human foetuses, neonates and adults. Eur J Clin Pharmacol 3:189–193 (1971).
34. Hamar C, Levy G. Serum protein binding of drugs and bilirubin in newborn infants and their mothers. Clin Pharmacol Ther 28:58–63 (1980).
35. Nau H, Luck W , Kuhnz W, Wegener S. Serum protein binding of diazepam, desmethyldiazepam, furosemide, indomethacin, warfarin, and phenobarbital in human fetus, mother, and newborn infant. Pediatr Pharmacol 3(3–4):219–227 (1983).
36. Mihaly GW, Morgan DJ. Placental drug transfer: effect of gestational age and species. Pharmacol Ther 23:253–266 (1984).
37. Hakkola J, Pelkonen O, Pasanen M. Xenobiotic-metabolizing cytochrome P450 enzymes in the human feto-placental unit: role in intrauterine toxicity. Crit Rev Toxicol 28(1):35–72 (1998).
38. Pratt, WB. The entry, distribution, and elimination of drugs. In: Principles of Drug Action, 3rd ed. (Pratt WB, Taylor P, eds). New York:Churchill Lingstone, 1990;201–296.
39. Morgan DJ. Drug disposition in mother and foetus. Clin Exp Pharmacol Physiol 24:869–873 (1997).
40. Ackerman H, Engst R. Presence of organophosphate insecticides in the fetus. Arch Toxicol 26:17–22 (1970).
41. Budreau CH, Singh RP. Transplacental passage of demeton in CF-1 mice. Arch Environ Health 26:161–163 (1973).
42. Harbison RD. Parathion-induced toxicity and phenobarbital-induced protection against parathion during prenatal development. Toxicol Appl Pharmacol 32:482–493 (1975).
43. Strother A, Wheeler L. Excretion and disposition of [^{14}C]carbaryl in pregnant, nonpregnant and foetal tissues of the rat after acute administration. Xenobiotica 10:113–124 (1980).
44. Berge GN, Nafstad I. Distribution and placental transfer of trichlorfon in guinea pigs. Arch Toxicol 59:26–29 (1986).
45. Hunter DL, Lassiter TL, Padilla S. Gestational exposure to chlorpyrifos: comparative distribution of trichloropyridinol in the fetus and dam. Toxicol Appl Pharmacol 158:16–23 (1999).
46. Mirkin BL, Singh S. Placental transfer of pharmacologically active molecules. In: Perinatal Pharmacology and Therapeutics (Mirkin BL, ed). New York:Academic Press, 1976;1–69.
47. Leiser R, Kaufmann P. Placental structure: in a comparative aspect. Exp Clin Endocrinol 102:122–134 (1994).
48. Goyer RA. Transplacental transport of lead. Environ Health Perspect 89:101–105 (1990).
49. Smit JW, Huisman MT, van Tellingen O, Wiltshire HR, Schinkel AH. Absence of pharmacological blocking of placental P-glycoprotein profoundly increases fetal drug exposure. J Clin Investig 104:1441–1447 (1999).
50. Maranghi F, Macri C, Ricciardi C, Stazi AV, Mantovani A. Evaluation of the placenta: suggestion for a greater role in developmental toxicology. Adv Exp Med Bio 444:129–136 (1998).
51. Firth JA, Farr A. Structural features and quantitative age-dependent changes in the intervascular barrier of the guinea-pig haemochorrial placenta. Cell Tissue Res 184(4):507–516 (1977).
52. Tzimas G, Collins M, Nau H. Developmental stage-associated differences in the transplacental distribution of 13-cis and all-trans-retinoic acid as well as their glucuronides in rats and mice. Toxicol Appl Pharmacol 133:91–101 (1995).
53. Garland M. Pharmacology of Drug Transfer Across the Placenta. Obstet Gynecol Clin N Am 25:21–42 (1998).
54. Pelkonen O. Detoxification and toxification processes in the

human feto-placental unit. In: Drugs and Pregnancy, Maternal Drug Handling-Fetal Drug Exposure (Krauer B, Krauer F, Hytten FE, del Pozo E, eds). London:Academic Press, 1984;63–72.
55. Rasmussen F. The mechanism of drug secretion into milk. In: Dietary Lipids and Postnatal Development (Galli C, Jacini G, Pecile A, eds). New York:Raven Press, 1973;231–245.
56. Wilson JT. Drugs in Breast Milk. Lancaster:MTP Press Ltd, 1981.
57. Wilson J, Brown R, Cherek D, Dailey J, Hilman B, Jobe P, Manno J, Redetzki H, Stewart J. Drug excretion in human breast milk: principles, pharmacokinetics and projected consequences. Clin Pharmacokinet 5:1–66 (1980).
58. Jenness R. The composition of human milk. Semin Perinatol 3:225–239 (1979).
59. Keen CL, Lonnerdal B, Clegg M, Hurley L. Developmental changes in composition of rat milk: trace elements, minerals, protein, carbohydrate and fat. J Nutr 111:226–236 (1981).
60. Berlin CM Jr. Pharmacologic considerations of drug use in the lactating mother. Obstet Gynecol 58(suppl 5):17S–23S (1981).
61. Berglund F, Flodh H, Lundborg P, Prame B, Sannerstedt R. Drug use during pregnancy and breast-feeding. Acta Obstet Gynecol Scand Suppl 126:1–55 (1984).
62. Rall DP. Secretion of foreign substances in breast milk. In: Breast Feeding and Food Policy in a Hungry World (Raphael D, ed). New York:Academic Press, 1979;233–240.
63. Rogan WJ, Bagniewska A, Damstra T. Pollutants in breast milk. N Engl J Med 302:1450–1453 (1980).
64. Sundberg J, Oskarsson A, Albanus L. Methylmercury exposure during lactation: milk concentration and tissue uptake of mercury in the neonatal rat. Bull Environ Contam Toxicol 46:255–262 (1991).
65. Hallen IP, Norrgren L, Oskarsson A. Distribution of lead in lactating mice and suckling offspring with special emphasis on the mammary gland. Arch Toxicol 70:237–243 (1996).
66. Petersson GK, Oskarsson A. Cadmium in milk and mammary gland in rats and mice. Arch Toxicol 73:519–527 (2000).
67. Albers J, Kreis I, Liem A, van Zoonen P. Factors that influence the level of contamination of human milk with polychlorinated organic compounds. Arch Environ Contam Toxicol 30:285–291 (1996).
68. Sinjari T, Klasson-Wehler E, Oskarsson A, Darnerud P. Milk transfer and neonatal uptake of coplanar polychlorinated biphenyl (PCB) congeners in mice. Pharmacol Toxicol 78:181–186 (1996).
69. Bagnell PC, Ellenberger HA. Obstructive jaundice due to a chlorinated hydrocarbon in breast milk. Can Med Assoc J 117:1047–1048 (1977).
70. Honour AJ, Myant NB, Rowlands EN. Secretion of radioiodine in digestive juices and milk in man. Clin Sci 11:447–463 (1952).
71. Spitzweg C, Joba W, Eisenmenger W, Heufelder A. Analysis of human sodium iodide symporter gene expression in extrathyroidal tissues and cloning of its complementary deoxyribonucleic acids from salivary gland, mammary gland, and gastric mucosa. Endocrinol Metab 83:1746–1751 (1998).
72. Courtney KD, Andrews JE. Neonatal and maternal body burdens of hexachlorobenzene (HCB) in mice: gestational exposure and lactational transfer. Fundam Appl Toxicol 5:265–277 (1985).
73. Ip HMH, Phillips DJH. Organochlorine chemicals in human breast milk in Hong Kong. Arch Environ Contam Toxicol 18:490–494 (1989).
74. Fleishaker J, Desai N, McNamara P. Factors affecting the milk to plasma concentration in lactating women: physical interactions with protein and fat. J Pharm Sci 76:189–193 (1987).
75. Fleishaker J, McNamara P. In vivo evaluation in the lactating rabbit of a model for xenobiotic distribution into breast milk. J Pharmacol Exp Ther 244:919–924 (1988).
76. Atkinson H, Begg E. The binding of drugs to major human milk whey proteins. Brit J Clin Pharmacol 26:107–109 (1988).
77. Begg EJ, Atkinson HC. Partitioning of drugs into human milk. Ann Acad Med Singap 20:51–55 (1991).
78. Banerjee NC, Miller GE, Stowe CM. Excretion of aminopyrine and its metabolites into cow's milk. Toxicol Appl Pharmacol 10:604–612 (1967).
79. Rasmussen F. Active mammary excretion of N4-acetylated *p*-aminohippuric acid. Acta Vet Scand 10:193–194 (1969).
80. Rasmussen F. Active mammary excretion of N4-acetylated sulphanilamide. Acta Vet Scand 10:402–403 (1969).
81. Lau R, Emery M, Galinsky R. Unexpected accumulation of acyclovir in breast milk with estimation of infant exposure. Obstet Gynecol 69:468–471 (1987).
82. McNamara PJ, Meece JA, Paxton E. Active transport of cimetidine and ranitidine into the milk of Sprague Dawley rats. J Pharmacol Exp Ther 277:1615–1676 (1996).
83. Somoji A, Gugler R. Cimetidine excretion into breast milk. Br J Clin Pharmacol 7:627–629 (1979).
84. Dostal LA. Investigation of the mechanisms of the extensive excretion of cimetidine into rat milk. Biochem Pharmacol 39:207–210 (1990).
85. Oo C, Kuhn RJ , Desai N, McNamara PJ. Active transport of cimetidine into human milk. Clin Pharmacol Ther 58:548–555 (1995).
86. Kari FW, Weaver R, Neville MC. Active transport of nitrofurantoin across the mammary epithelium *in vivo*. J Pharmacol Exp Ther 280:664–668 (1997).
87. Neville MC, Walsh CT. Effects of xenobiotics on milk secretion and composition. Am J Clin Nutr 61:687S–694S (1995).
88. Hamshaw-Thomas A, Rogerson N, Reynolds F. Transfer of bupivacaine, lignocaine and pethidine across the rabbit placenta: influence of maternal protein binding and fetal flow. Placenta 5:61–70 (1984).
89. Golub MS, Eisele JH. Maternal-fetal distribution of morphine and alfentanil in near-term sheep and rhesus monkeys. Dev Pharmacol Ther 9:12–22 (1986).
90. Laishley RS, Carson RJ, Reynolds F. Effect of adrenaline on placental transfer of bupivacaine in the perfused *in situ* rabbit placenta. Br J Anaesth 63(4):439–443 (1989).
91. Carson RJ, Reynolds F. Maternal-fetal distribution of bupivacaine in the rabbit. Br J Anaesth 61(3):332–337 (1988).
92. Michiels M, Hendriks R, Heykants J. On the pharmacokinetics of domperidone in animals and man. II: Tissue distribution, placental and milk transfer of domperidone in the Wistar rat. Eur J Drug Metab Pharmacokinet 6:37–48 (1981).
93. Creech-Kraft J, Lofberg B, Chahoud I, Bochert G, Nau H. Teratogenicity and placental transfer of all-trans-, 13-cis-, 4-oxo-all-trans-, and 4-oxo-13-cis-retinoic acid after administration of a low oral dose during organogenesis in mice. Toxicol Appl Pharmacol 100:162–176 (1989).
94. Nau H, Scott W. Weak acids may act as teratogens by accumulating in the basic milieu of the early mammalian embryo. Nature 323:276–278 (1986).
95. O'Flaherty EJ, Scott W, Schreiner C, Beliles RP. A physiologically based kinetic model of rat and mouse gestation: disposition of a weak acid. Toxicol Appl Pharmacol 112:245–256 (1992).
96. Byczkowski JZ, Fisher JW. Lactational transfer of tetrachloroethylene in rats. Risk Analysis 14:339–349 (1994).
97. Stevens M, Harbison R. Placental transfer of diphenylhydantoin: effects of species, gestational age and route of administration. Teratology 9:317–326 (1974).
98. Bazare JJ, Nelson CJ, Young JF. Pharmacokinetics of 2,4,5-T in mice as a function of dose and gestational status. Reprod Toxicol 4:137–144 (1990).
99. Chou RC, Levy G. Effect of pregnancy on the pharmacokinetics of phenytoin rats. J Pharmacol Exp Ther 229:351–358 (1984).
100. Kobayashi S, Takai K, Iga T, Hannano M. Pharmacokinetic analysis of the disposition of valproate in pregnant rats. Drug Metab Dispos 19:972–976 (1991).
101. Kimmel CA, Young JF. Correlating pharmacokinetics and teratogenic endpoints. Fundam Appl Toxicol 3:250–255 (1983).
102. Nau H. Species differences in pharmacokinetics and drug teratogenesis. Environ Health Perspect 70:113–129 (1986).
103. Wegner C, Nau H. Diurnal variation of folate concentrations in mouse embryo plasma: the protective effect of folic acid on valproic-acid-induced teratogenicity is time dependent. Reprod Toxicol 5:465–471 (1991).
104. Nau H. Teratogenic valproic acid concentrations: infusion by implanted minipumps vs. conventional injection regimen in the mouse. Toxicol Appl Pharmacol 80:243–250 (1985).
105. Nau H. Correlation of transplacental and maternal pharmacokinetics of retinoid during organogenesis with teratogenicity. Methods Enzymol 190:437–448 (1990).
106. Laughlin NK. Animal models of behavioral effects of early lead exposure. In: Handbook of Behavioral Teratology (Riley EP, Vorhees CV, eds). New York:Plenum Press, 1986;291–319.
107. Terry K, Elswick B, Stedman D, Welsch F. Developmental phase alters dosimetry-teratogenicity relationship for 2-methoxymethanol in CD-1 mice. Teratology 49:218–227 (1994).
108. Clarke DO, Duignan JM, Welsch F. 2-methoxyacetic acid dosimetry-teratogenicity relationships in CD-1 mice exposed to 2-methoxyethanol. Toxicol Appl Pharmacol 114:77–87 (1992).
109. Nau H. Percutaneous retinoid absorption and embryotoxicity. J Investig Dermatol 97:159–160 (1991).
110. Sundberg J, Jonsson S, Karlsson M, Oskarsson A. Lactational exposure and neonatal kinetics of methylmercury and inorganic mercury in mice. Toxicol Appl Pharmacol 154:160–169 (1999).
111. Welsch F, Blumenthal G, Conolly R. Physiologically based pharmacokinetics models applicable to organogenesis: extrapolation between species and potential use in prenatal toxicity risk assessments. Toxicol Lett 82/83:539–547 (1995).
112. Clarkson TW. The role of biomarkers in reproductive and development toxicology. Environ Health Perspect 74:103–107 (1987).
113. Morse DC, Plug A, Wesseling W, van den Berg KJ, Brouwer A. Persistent alterations in regional brain glial fibrillary acidic protein and synaptophysin levels following pre- and postnatal polychlorinated biphenyl exposure. Toxicol Appl Pharmacol 139(2):252–261 (1996).
114. Sloviter R, Lowenstein D. Heat shock protein expression in vulnerable cells of the rat hippocampus as an indicator of excitation-induced neuronal stress. J Neurosci 12:3004–3009 (1992).
115. Costa, LG. Effects of neurotoxicants on brain neurochemistry. In: Neurotoxicology (Tilson H, Mitchell C, eds). New York:Raven Press, 1992;101–124.
116. Whitney K, Seidler F, Slotkin TA. Developmental neurotoxicity of chlorpyrifos: cellular mechanisms. Toxicol Appl Pharmacol 134:53–62 (1995).
117. Royland JE, Konat G, Wiggins RC. Abnormal upregulation of myelin genes underlies the critical period of myelination in undernourished developing rat brain. Brain Res 607:113–116 (1993).
118. Dvergsten C, Meeker RB. Muscarinic cholinergic receptor regulation and acetylchlinesterase inhibition in response to insecticide exposure during development. Int J Dev Neurosci 12:63–75 (1994).
119. Clarke C, Clarke K, Muneyyirci J, Azmitia E, Whitaker-Azmitia PM. Prenatal cocaine delays astroglial maturation: immunodensitometry shows increased markers of immaturity (vimentin and GAP-43) and decreased proliferation and production of the growth factor S-100. Brain Res Dev 91:268–273 (1996).
120. Zoeller R, Dowling A, Vas A. Developmental exposure to polychlorinated biphenyls exerts thyroid hormone-like effects on the expression of RC3/neurogranin and myelin basic protein messenger ribonucleic acids in the developing rat brain. Endocrinology 141:181–189 (2000).
121. Munoz A, Rodriguez-Pena A, Perez-Castillo A, Ferreiro B, Sutcliffe JG, Bernal J. Effects of neonatal hypothyroidism on rat brain gene expression. Mol Endocrinol 5:273–280 (1991).
122. Tandon P, Pope C, Padilla S, Tilson H, Harry GJ. Developmental changes in carbachol-stimulated inositol phosphate release in pigmented rat retina. Curr Eye Res 12:439–449 (1993).
123. Song X, Seidler F, Zhang J, Padilla S, Slotkin TA. Cellular mechanisms for developmental toxicity of chlorpyrifos: targeting the adenylyl cyclase signaling cascade. Toxicol Appl Pharmacol 134:158–174 (1997).
124. Selvin-Testa A, Capani F, Loidl CF, Lopez EM, Pecci-Saavedra J. Prenatal and Postnatal lead exposure induces 70 kDA heat shock protein in young rat brain prior to changes in astrocyte sytoskeleton. Neurotoxicology 18(3):805–817 (1997).
125. Nuwaysir EF, Bittner M, Trent J, Barrett JC, Afshari CS. Microarrays and toxicology: the advent of toxicogenomics. Mol Carcinog 24(3):153–159.
126. Gerlowski LE, Jain RK. Physiologically based pharmacokinetic modeling: principles and applications. J Pharm Sci 72:1103–1127 (1983).
127. Andersen M. Physiological modeling of organic compounds. Ann Occup Hyg 35:309–321 (1991).
128. Fisher JW, Whittaker TA, Taylor DH, Clewell HJ, Andersen ME. Physiologically based pharmacokinetic modeling of the pregnant rat: A multiroute exposure model for trichloroethylene and its metabolite, trichloroacetic acid. Toxicol Appl Pharmacol 99:395–414 (1989).
129. Clarke DO, Elswick BA, Welsch F, Conolly RB. Pharmacokinetics of 2-methoxyethanol and 2-methoxyacetic acid in the pregnant mouse: a physiologically based mathematical model. Toxicol Appl Pharmacol 121:239–252 (1993).
130. Gray DG. A Physiologically based pharmacokinetic model for methyl mercury in the pregnant rat and fetus. Toxicol Appl Pharmacol 132:91–102 (1995).
131. Terry K, Elswick B, Welsch F, Conolly R. Development of a physiologically based pharmacokinetic model describing 2-methoxyacetic acid disposition in the pregnant mouse. Toxicol Appl Pharmacol 132:103–114 (1995).
132. Ward K, Blumenthal G, Welsch F, Pollack G. Development of a physiologically based pharmacokinetic model to describe the disposition of methanol in pregnant rats and mice. Toxicol Appl Pharmacol 145:311–322 (1997).
133. Byczkowski JZ, Kinkead ER, Leahy HF, Randall GM, Fisher JW. Computer simulation of the lactational transfer of tetrachloroethylene in rats using a physiologically based model. Toxicol Appl Pharmacol 125:228–236 (1994).
134. Fisher J, Mahle D, Bankston L, Greene R, Gearhart J. Lactational transfer of volatile chemicals in breast milk. Am Ind Hyg Assoc J 58:425–431 (1997).
135. Omtzigt JGC, Nau H, Los FJ, Pijpers L, Lindhout D. The disposi-

tion of valproate and its metabolites in the late first trimester and early second trimester of pregnancy in maternal serum, urine, and amniotic fluid. J Clin Pharmacol 43:381–388 (1992).
136. Wittfoht W, Nau H, Rating D, Helge H. ^{13}C-Labelled valproic acid pulse dose during steady state antiepileptic therapy for pharmacokinetic studies during pregnancy. Adv Exp Med Biol (1982).
137. Nau H, Rating D, Koch S, Hauser I, Helge H. Valproic acid and its metabolites: placental transfer, neonatal pharmacokinetics, transfer via mother's milk and clinical status in neonates of epileptic mothers. J Pharmacol Exp Ther 219:768–777 (1981).
138. U.S. Environmental Protection Agency. Guidelines for exposure assessment. Fed Reg 57:22888–22938 (1992).
139. Brown RP, Delp MD, Linstedt SL. Physiological parameter values for physiologically based pharmacokinetic models. Toxicol Ind Health 13:407–484 (1997).
140. Bennett GD, Finnell RH. Periods of susceptibility to induced malformations of the developing mammalian brain. In: Handbook of Developmental Neurotoxicology (Slikker W, Chang LW, eds). New York:Academic Press, 1998;189–208.
141. Lauder JM, Schambra UB. Morphogenetic roles of acetylcholine. Environ Health Perspect 107(suppl 1):65–69 (1999).
142. Slotkin T. Developmental cholinotoxicants: nicotine and chlorpyrifos. Environ Health Perspect 107(suppl 1):71–80 (1999).
143. Francis EZ. Testing of environmental agents for developmental and reproductive toxicity. In: Developmental Toxicology, 2nd ed. (Kimmel CA, Buelke-Sam J, eds). New York:Raven Press, Ltd., 1994;403–428.
144. Golub MS. Adolescent health and the environment. Environ Health Perspect 108(suppl 4):355–362 (2000).
145. Thompson P, Giedd J, Woods R, MacDonald D, Evans A, Toga A. Growth patterns in the developing brain detected by using continuum mechanical tensor maps. Nature 404:190–193 (2000).
146. Jacobson M, ed. The germinal cell, histogenesis, and lineages of nerve cells. In: Developmental Neurobiology, 3rd ed. New York:Plenum Press, 1991;41–93.
147. Totsuka S, Sugano A, Manabe S, Mori M. Study on the repeated oral administration method in infant rats. Jikken Dobutsu 38:17–24 (1989).
148. Dorman DC, Struve MF, Vitarella D, Byerly FL, Goetz J, Miller R. Neurotoxicity of manganese chloride in neonatal and adult CD rat following subchronic (21-day) high-dose oral exposure. J Appl Toxicol 20:179–187 (2000).
149. Hayden LJ, Goeden H, Roth SH. Growth and development in the rat during sub-chronic exposure to low levels of hydrogen sulfide. Toxicol Ind Health 6:389–401 (1990).
150. Levin ED, DeLuna R, Uemura E, Bowman RE. Long-term effects of developmental halothane exposure on radial arm maze performance in rats. Behav Brain Res 36:147–154 (1990).
151. Stern S, Cox C, Preston R, Sharma A, Inglis GB, Balys M, Weiss B. Perinatal methanol exposure in the rat. II. Behavioral effects in neonates and adults. Fundam Appl Toxicol 36(2):163–176 (1997).
152. Vitarella D, Wong B, James R, Miller K, Struve M, Dorman DC. Development of an inhalation system for the conduct of developmental neurotoxicity studies. Inhal Toxicol 10:1095–1117 (1998).
153. Dorman DC, Brenneman KA, Struve MF, Miller KL, James RA, Marshall MW, Foster PMD. Fertility and developmental neurotoxicity effects of inhaled hydrogen sulfide in Sprague-Dawley rats. Neurotoxicol Teratol 22:71–84 (2000).
154. Miller ST, Cotzias GC, Evert HA. Control of tissue manganese: initial absence and sudden emergence of excretion in the neonatal mouse. Am J Physiol 229:1080–1084 (1975).
155. Oskarsson A, Palminger HI, Sundberg J, Petersson GK. Risk assessment in relation to neonatal metal exposure. Analyst 123:19–23 (1998).
156. Benke GM, Murphy SD. The influence of age on the toxicity and metabolism of methyl parathion and parathion in male and female rats. Toxicol Appl Pharmacol 31:254–269 (1975).
157. Lu FC, Jessup DC, Lavallee A. Toxicity of pesticides in young versus adult rats. Food Cosmet Toxicol 3:591–596 (1965).
158. Mortensen SR, Chanda SM, Hooper MJ, Padilla S. Maturational differences in chlorpyrifos-oxonase activity may contribute to age-related sensitivity to chlorpyrifos. J Biochem Toxicol Sci 11:279–287 (1996).
159. Atterberry T, Burnett W, Chambers J. Age-related difference in parathion and chlorpyrifos toxicity in male rats: target and nontarget esterase sensitivity and cytochrome P450-mediated metabolism. Toxicol Appl Pharmacol 147:411–418 (1997).
160. Moser VC, Chanda SM, Mortensen SR, Padilla S. Age- and gender-related differences in sensitivity to chlorpyrifos in the rat reflect development profiles of esterase activity. Toxicol Sci 46:211–222 (1998).
161. Andersen H, Nielson J, Granjean P. Toxicologic evidence of developmental neurotoxicity of environmental chemicals. Toxicology 144:121–127 (2000).
162. Smith Q. Transport of glutamate and other amino acids at the blood–brain barrier. J Nutr 130(suppl 4S):16s–22s (2000).
163. Bauer H-C, Bauer H. Neural induction of the blood–brain barrier still an enigma. Cell Mol Neurobiol 20(1):13–28 (2000).
164. Saunders NR, Knott GW, Dzeigielewska KM. Barriers in the immature brain. Cell Mol Neurobiol 20(1):29–40 (2000).
165. Mollgard K, Saunders NR. The development of the human blood–brain and blood–CSF barriers. Neuropathol Appl Neurobiol 12:337–358 (1986).
166. Risau W. Differentiation of endothelium. FASEB J 9:925–933 (1995).
167. Risau W, Wolberg H. Development of the blood–brain barrier. Trends Neurosci 13(5):174–178 (1990).
168. Claudio L, Kwa W, Russell A, Wallinga D. Testing methods for developmental neurotoxicity of environmental chemicals. Toxicol Appl Pharmacol 164(1):1–14 (2000).
169. Habgood M, Knott G, Dzeigielewska KM, Saunders NR. Permeability of the developing and mature blood–brain barriers to theophylline in rats. Clin Exp Pharmacol Physiol 25(5):361–368 (1998).
170. Boylan J, Egle J, Guzelian P. Cholestyramine: use as a new therapeutic approach for chlordecone (kepone) poisoning. Science 199(4331):893–895 (1978).
171. Rozman KK, Klaassen CD. Absorption, distribution and excretion of toxicants. In: Casarett & Doull's Toxicology: The Basic Science of Poisons, 5th ed. (Klaassen C, ed). New York:McGraw-Hill, 1996;91–112.
172. Webb M, Holt D. Endogenous metal binding proteins in relation to the differences in absorption and distribution of mercury in newborn and adult rats. Arch Toxicol 49(3–4):237–245 (1982).
173. Dishino DD, Welch MJ, Kilbourn MR, Raichle ME. Relationship between lipophilicity and brain extraction of C-11 labeled radiopharmaceuticals. J Nucl Med 24:1030–1038 (1983).
174. Banks WA, Kastin AJ. Permeability of the blood-brain barrier to neuropeptides: the case for penetration. Psychoneuroendocrinology 10:385–399 (1985).
175. Pardridge WM. Recent advances in blood-brain barrier transport. Annu Rev Pharmacol Toxicol 28:25–39 (1988).
176. Malecki EA, Devenyi AG, Beard JL, Connor JR. Existing and emerging mechanisms for transport of iron and manganese to the brain. Neurosci Res 56:113–122 (1999).
177. Takeda A, Ishiwatari S, Okada S. Influence of transferrin on manganese uptake in the rat brain. Neurosci Res 59:542–552 (2000).
178. Hill JM, Ruff MR, Weber RJ, Pert CB. Transferrin receptors in rat brain: neuropeptide-like pattern and relationship to iron distribution. Proc Natl Acad Sci U S A 82:4553–4557 (1985).
179. Morris CM, Candy JM, Oakley AE, Taylor GA, Mountfort S, Bishop H, Ward MK, Bloxham CA, Edwardson JA. Comparison of the regional distribution of transferrin receptors and aluminium in the forebrain of chronic renal dialysis patients. J Neurol Sci 94:295–306 (1989).
180. Tsuchiya K. Lead. In: Handbook on the Toxicology of Metals (Friberg L, Nordberg GF, Vouk VB, eds). New York:Elsevier, 1979;451–484.
181. Page KR. The Physiology of the Human Placenta. London: University College of London, 1993;13.